REMARKS

Claims 13-15 have been cancelled. New Claims 24-33 have been added to more clearly define the invention. Support for these new claims can be found throughout the specification, including on page 11, line 10, to page 13, line 15; and Figure 1. Accordingly, Claims 24-33 are pending.

Additionally, some typographical errors have been corrected in the specification. No new matter has been introduced.

Present Invention

The present invention provides a novel method for labelling bio-organic molecules. The method allows for a greater number of distinct labels to be generated from a fixed number of stains, paints or dyes, e.g., fluorophores, than have been generated from prior art methods.

In the present specification, the term "colour" is not used in the conventional sense. That is, the term "colour" is not used merely to mean a monochromatic pigment.

Instead, the term "colour" is used to mean a distinct label. Labels may be distinct from one another due to the presence of different mixtures of monochromatic pigments. For example, such pigments may result from the mixing together of different stains, paints or dyes. Additionally, labels may be distinct from one another due to the presence of different monochromatic pigments side by side. Further, labels may be distinct from one another due to the presence of a hapten, or immunogenic/antigenic determinant.

The present method can be used, for example, to selectively label bio-organic molecules, such as chromosomes. Such labelling has many applications, including but not

limited to, cytogenetics; cancer research; genomics; proteomics; drug discovery and delivery; and food and feed technology.

Before the present method, selective labelling of chromosomes has typically been accomplished by one of two methods, either binary labelling or ratio labelling

Binary labelling uses combinations of probes, wherein each individual probe is associated with a distinct label, for example, a distinct fluorophore. Binary labelling is also called combinatorial labelling. Each combination is targeted to a specific bio-organic molecule, for example, a chromosome.

The number of distinct "colours" (n) using k different binary labels is $n=2^k-1$. In the formula, 1 is subtracted from 2^k so as to remove the combination where red, blue and yellow are all absent.

As can be seen in the table, for example, three fluorophores would generate a maximum of 7 "colours."

	COLOUR						
Fluorophore	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Blue	+	+	+	-	-	+	-
Red	+	+	-	-	+	-	+
Yellow	+	-	-	+	+	+	-

In the table, a "+" indicates the presence of a fluorophore; and a "-" indicates the absence of a fluorophore.

Thus, five fluorophores would allow a maximum of 31 "colours" ($31=2^5-1$) by binary labelling. Thirty-one "colours" are sufficient to distinctly stain the twenty-four human chromosomes.

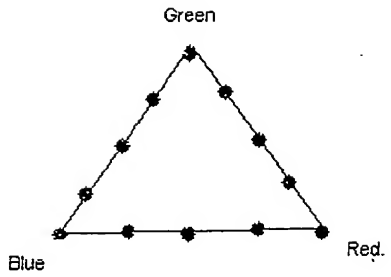
Ratio labelling, by contrast, is based on the *quantity* of each dye present in a mixture of dyes, i.e. signal strength. A certain number of dyes, for example two fluorophores, taken from a pool of spectrally distinct fluorophores, are simultaneously used to produce a certain "colour" taking into account the quantity of each fluorophore. That is, the relative presence (i.e. signal strength) of each fluorophore is assessed.

For example, using a three fluorophore pool, allowing two fluorophores to simultaneously produce a certain "colour," and resolving three distinct ratios for each pair of fluorophores, ratio-labelling provides 12 "colours." The table below provides an illustration.

	COLOUR												
Fluorophore	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
Green	50	25	75	50	25	75	-	-	-	100	-	-	
Red	-	-	-	50	75	25	50	75	25	-	100	-	
Blue	50	75	25	-	-	-	50	25	75	-	-	100	

As explained above, a "colour" can be viewed as a combined presence of dyes, not necessarily a physical mixture of dyes. Thus, for example, Colour 8 in the table can be viewed as the dual presence of a red fluorophore and a blue fluorophore in a ratio of 75:25, not necessarily as purple. Alternatively, instruments may be set so as to also view the "colour" as a monochromatic mixture of the red and blue fluorophores.

Level 0 of Figure 1 of the application illustrates this table in graphical form. Level 0 of the figure is roughly reproduced below.

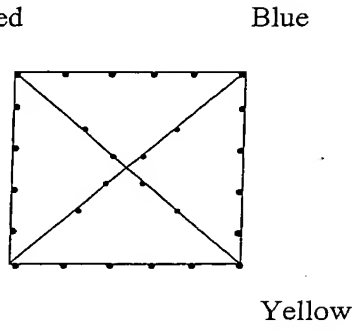


Each point on the triangle represents a distinct "colour." The points at each vertex of the triangle represent a "colour" generated solely from a particular fluorophore (Colours 10, 11, and 12 in the table). The midpoint of each side of the triangle represents a "colour" which is generated from a 50% presence of each of two fluorophores. For example, the midpoint of the left side represents a "colour" which is 50% blue and 50% green (Colour 1 in the table). Moving up one point represents a "colour" which is 75% green and 25% blue (Colour 3 in the table).

Please note that each side of the triangle has four segments to represent ratios in ¼ fractional increments. However, the quantities of two dyes making up a probe may be distinguishable at ratios using lower fractional increments. For example, for ratios using $^1/_5$ fractional increments, each side of the triangle would be divided into five segments. Ratio labelling is limited by the lowest fractional presence of a dye in a probe, vis-à-vis the other dyes in the same probe, that can be distinctly resolved.

The illustration above is based on a pool of three dyes. A pool of dyes, i.e. a fluorophore pool, may, however, contain more than three dyes.

For example, using a four fluorophore pool, allowing two fluorophores to simultaneously produce a certain "colour," and resolving four distinct ratios for each pair of fluorophores, ratio-labelling provides 28 "colours." The square shown below provides an illustration. Each dot represents a "colour."



Also, more than two dyes can simultaneously be used to produce a certain "colour." For example, using a three fluorophore pool, all three fluorophores can be used simultaneously to produce a certain "colour." For example, a green, blue and red fluorophore can each have a 1/3 presence in a certain "colour."

The number of "colours" provided by binary or ratio labelling is insufficient for many applications. As mentioned above, for example, using five dyes, binary labelling provides 31 "colours." Using five dyes, wherein two dyes are used simultaneously to generate each "colour," and distinguishing three ratios, ratio labelling provides 35 "colours." Such a limited number of "colours" provided by these two methods would be insufficient, for example, to distinctly label intrachromosomal rearrangements.

It would thus be necessary to utilize additional dyes to distinctly label such via binary labelling or ratio labelling. The use of additional dyes can be cumbersome, complicated, and expensive. Therefore, the labelling of bio-organic molecules, such as chromosomes, would greatly benefit from a method to increase the number of simultaneously recognizable "colours" producible from a small number of dyes.

The present invention provides a method for generating a greater number of "colours" from a fixed number of different dyes than by the prior art methods. The method combines

binary labelling with ratio labelling to provide combined binary ratio labelling, i.e., COBRA labelling.

In the COBRA labelling method, a first set of primary probes is labelled via ratio labelling with distinct "colours." This probe set is directed to a first set of targets.

At least one other set of primary probes, directed to a second set of targets, is ratio labelled in exactly the same way as the first primary probe set. However, this second primary probe set additionally contains a binary label, which is spectrally well distinguished from the ratio labels, or contains a biological determinant, e.g., a hapten. This binary label is considered to transform each of the "colours" generated by the ratio labelling of the second primary probe set into an additional distinct "colour." Thus, the addition of the binary label doubles the number of distinct labels obtained by ratio-labelling.

Figure 1 of the application provides a graphical representation of this concept. A copy is enclosed for the Examiner's convenience. Here, ratio labelling is accomplished by using a three dye pool from which two dyes are used simultaneously to generate each "colour." When no binary label is used (level 0), one set of 12 "colours" is shown to be possible from the three dyes. When one binary label is used (level 1), two sets of 12 "colours" are shown. One set is accompanied by a white rectangle; the second set is accompanied by a red rectangle. The red rectangle represents the binary label. The white rectangle represents the absence of a binary label. Thus, the binary label doubles the number of "colours" provided by the three dyes. Keep in mind that a "colour" as defined in the specification is simply a distinct label.

When two binary labels are used (level 2 in figure 1), four sets of the 12 "colours," i.e. 48 "colours," are generated. Thus, with the addition of each binary label, the number of "colours" producible from a fixed number of dyes doubles.

Using only two dyes simultaneously per "colour," the total number of achievable COBRA "colours" (I) can mathematically be described as:

$$I = (n + ((r \times n!) / (2 \times (n - 2)!))) \times 2^m$$

wherein n is the number of dyes used for ratio labelling, m is the number of labels used for binary labelling, and r is the number of ratios that is resolved by ratio labelling; and wherein

$$2 \leq n \leq \infty,$$
$$0 \leq r \leq \infty$$
$$0 \leq m \leq \infty$$

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This application is now in condition for examination on the merits. Such examination at the Examiner's earliest convenience is respectfully requested.

Respectfully submitted,

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